QUANTITATIVE STUDY ON THE REGIONAL DISTRIBUTION OF PENTOSE NUCLEIC ACID IN THE GASTRULA AND NEURULA OF TRITURUS

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The aim of the present study is to estimate chemically the amount of pentose nucleic acid in the different prospective regions of the ectoderm and mesoderm in the early gastrula and the early neurula of Amphibia, and to obtain quantitative information about its spatial distribution and its change during gastrulation. The eggs of *Triturus pyrrhogaster,* which were found to satisfy special demands of our study, were used exclusively. In this material the prospective significance and the morphogenetic movement of the different regions have been worked out in detail (Nakamura, 1942) and operative separation of these regions can be successfully carried out with techniques developed in this laboratory.

Material and Method

The estimation was carried out on the following developmental stages: (1) gastrula with beginning blastopore, corresponding approximately to stage 11 of Okada and Ichikawa's table of *Triturus pyrrhogaster* (1947) and to stage 10 of Harrison's table of *Amblystoma punctatum;* (2) neurula, shortly after the formation of the neural folds, corresponding to stage 16 of Okada and Ichikawa's table and to stage 14 of Harrison's table.

Following the removal of the capsule and vitelline membrane the embryos were operated on in Holtfreter's solution on a Schotté ring using the glass needle and glass spherule. For a better separation of the germ layers the operation dish was kept cold with ice. During the operation utmost care was taken to obtain isolates of pure prospective significance, free from contamination by cells from the adjoining regions and underlying layer.

In the gastrula the following regions were isolated for estimation:

(a) The prospective ectoderm (A, Fig. 1a), consisting of the prospective neural plate (the cross-hatched part of A) and the prospective epidermis (the plain part of A). In a special series the prospective neural plate and the prospective epidermis were estimated separately.

(b) The dorsal marginal zone (B, Fig. 1a), containing the prospective notochord and the dorsal half of the prospective somites. The prospective pre-

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chordal material, which is continuous with the yolk-rich endoderm of the pharyngeal wall, without a clear boundary, was not included.

(c) The lateral marginal zone (C, Fig. 1a), containing the ventral part of the prospective somites and the prospective lateral plate.

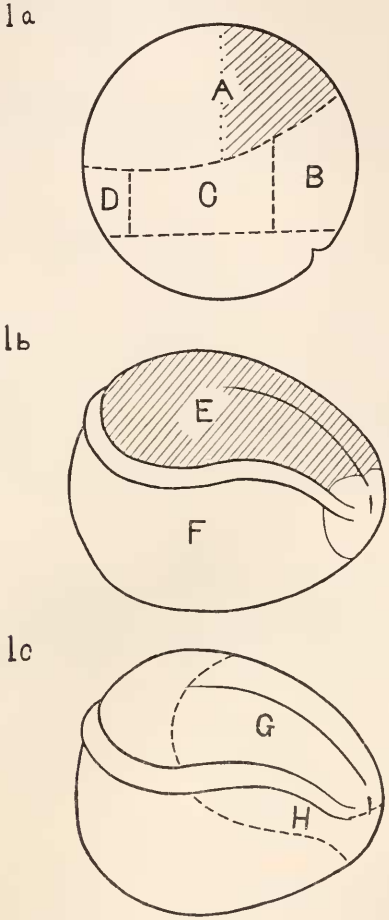

FIGURE 1. Schemes for isolation of samples for PNA estimation. 1a: Gastrula; A: prospective ectoderm (the cross-hatched half is the prospective neural plate, the plain half the prospective epidermis); B: dorsal marginal zone; C: lateral marginal zone; D: ventral marginal zone. 1b: Neurula; E: neural plate (cross-hatched area); F: epidermis (neural fold, and area surrounding the blastopore are omitted). 1c: Neurula; G: dorsal mesoderm; H: lateral and ventral mesoderm.

(d) The ventral marginal zone (D, Fig. 1a), containing the prospective blood island and adjoining ventral mesoderm.

From the neurula the following regions were estimated:

(a) The neural plate (E, Fig. 1b). The neural folds were not included. Also the most caudal one-fifth of the neural plate which contained the prospective

somites (Nakamura, 1942) was omitted. The cells of the archenteric roof, which were sticking to the internal surface of the neural plate, were removed very carefully with the finest glass spherules.

(b) The epidermis (F, Fig. 1b). Only very careful operation gave pure epidermis without attached mesoderm cells, which were more difficult to remove than the archenteric roof cells. Of course only pure isolates were used for the estimation.

(c) The archenteric roof (G, Fig. 1c). Most of the prechordal plate was omitted. No endodermal pharyngeal wall was included. This region corresponded to region B and the dorsal part of region C of the gastrula.

(d) The latero-ventral mesoderm (H, Fig. 1d); the mesoderm underlying the epidermis. This is derived from region D and the ventral part of region C of the gastrula.

For the chemical estimation of PNA, Schneider's technique (1945) was adopted. The intensity of orcinol reaction was read by a Pulfrich spectrophotometer. Interference by DNA contained in the "PNA-fraction" was found to be negligible. PNA concentration was expressed in micrograms of phosphorus per milligram of the total nitrogen. Total nitrogen was estimated by the method of Levy and Palmer (1940) on the residue after the nucleic acid extraction. In the principal series six separate determinations were carried out for each region of both stages, while in the additional series four separate determinations were made. Thirty-five to 45 pieces of isolates made up the sample for one estimation.

TABLE I

PNA Pγ per mg. N of the different prospective regions of the early gastrula and the early neurula

$\bar{X}$: mean; $S\bar{x}$: standard deviation of the mean; **: F_0-value significant at 1% level; *: F_0-value significant at 5% level but not so at 1% level; °: the same value not significant on 5% level.

Stage	Prospective regions	PNA Pγ/mg. N $\bar{X} \pm S\bar{x}$	Ratio of variances F_0
Gastrula	(A) Prosp. ectoderm	9.6±0.60	28.93**
	(B) Dorsal marginal zone	6.0±0.30	11.42*
	(C) Lateral marginal zone	4.8±0.11	0.25°
	(D) Ventral marginal zone	4.6±0.33	
	(A') Prosp. neural plate	11.5±0.28	10.98*
	(A'') Prosp. epidermis	9.7±0.47	
Neurula	(E) Neural plate	13.8±0.68	7.88*
	(F) Epidermis	11.1±0.66	11.83**
	(G) Dorsal mesoderm	7.7±0.73	0.01°
	(H) Latero-ventral mesoderm	7.6±0.74	

TABLE II

Comparison of PNA Pγ/mg. N of prospective regions of the gastrula and neurula. Compare with Table I

Prospective regions	Ratio of variances F_0
Prospective neural plate of gastrula Neural plate of neurula	6.9*
Prospective epidermis of gastrula Epidermis of neurula	2.67°
Prospective ectoderm of gastrula Neural plate of neurula	20.67**
Prospective ectoderm of gastrula Epidermis of neurula	2.83°

RESULTS

The principal data obtained are summarized in Table I.

Gastrula. The prospective ectoderm (A) showed the highest value, while the dorsal marginal zone (B) gave a value significantly less than the former. The dorsal marginal zone, the organizer region, in its turn showed PNA content higher than the lateral and ventral sections of the marginal zone. This difference was significant at the 5% level, but hardly so at the 1% level. The latter two sections failed to give any significant difference.

In the additional series PNA content of the prospective neural plate and the prospective epidermis was estimated (Table II). The results showed a difference significant at the 5% level in favor of the prospective neural plate.

Neurula. The neural plate (E) showed the highest value, significantly higher than the dorsal and latero-ventral mesoderm of the same stage (G and H). The difference between the neural plate (E) and the epidermis (F) was found to be significant at the 5% level, but not at the 1% level. No significant difference could be detected between the dorsal and latero-ventral mesoderm.

DISCUSSION

The present data show clearly that in both stages examined the ectoderm contains more PNA than the mesoderm, if the amount is calculated on the over-all nitrogen base. The organizer region, *i.e.*, the dorsal marginal zone of the gastrula and the archenteric roof of the neurula, contain significantly less PNA than is contained in the ectoderm in both stages. Within the ectoderm the neural plate and its prospective region seem to possess more PNA than the epidermis and its prospective region, although the difference obtained is significant only at the 5% level. Thus, our data make it very probable that the apex of the gradient of the substance throughout both germ layers is present in the material of the neural system in the stages studied. Comparing the data on ectodermal regions of both stages it might be pointed out that during gastrulation an accumulation significant at the 5% level can be detected for the neural plate region, but not for the epidermis region.

As to the marginal zone our data show a difference significant at the 5% level

between its dorsal section and the rest of the zone in the gastrula. However, in the neurula no significant difference can be shown between the dorsal and latero-ventral mesoderm.

If we compare the data obtained from different regions with the inductive effect of these regions on the presumptive ectoderm, it is clear that the simple over-all PNA concentration of any germ region as such does not decide the inductive capacity of that region. The epidermis of the neurula is non-inductive and the ectoderm of the gastrula is very weakly if at all inductive, but they contain more PNA than the organizer itself. In the early neurula the archenteric roof has stronger inductive effect but not the latero-ventral mesoderm. However, they all have about the same amount of PNA. Furthermore this amount is decisively less than that of the non-inductive epidermis.

As to the spatial distribution of PNA within the different prospective regions of the amphibian embryo, until now only data based on the observation of the histochemical section have been available. The general features of the gradient as demonstrated by histochemical techniques by Brachet (1942, 1947), such as the animal-vegetal and dorso-ventral gradient, are mostly in accord with our present data. However, no indication was obtained in the present study of a high concentration of PNA in the organizer. The present author studied the PNAase-sensitive basophilia in embryos of *Rhacophorus schlegelii* (anura, pigmentless eggs; Takata, 1950), *Triturus pyrrhogaster* and *Megalobatrachus japonicus* (urodele, pigmentless eggs; Takata, unpublished results). During gastrulation the apex of the gradient was found always in the prospective neural plate. The dorsal marginal zone was stronger in intensity than the lateral and ventral marginal zone, but decisively weaker than the ectodermal regions. These observations are in good accord with the present data and there seems to be no ground to support the idea that the organizer represents the apex of the PNA gradient, if its value is calculated on the basis of over-all nitrogen. However, it is still an undecided question whether the PNA concentration of the active protoplasm (*i.e.,* PNA calculated on the basis of the non-yolk nitrogen) has its highest value in the organizer region.

Insofar as data on total nitrogen basis are concerned we did not find any indication for decrease of PNA in the invaginating archenteric roof, as suggested by Brachet from his histochemical sections.

Comparing the data on two stages, a general increase of PNA content in both layers during gastrulation is evident. This rise seems to be in accord with the data on the total PNA amount of amphibian embryos during the early development, where more or less clear increase of the value beginning from gastrulation was noticed (Brachet, 1941; Steinert, 1951).

Summary

1. PNA content of prospective regions of the ectoderm and mesoderm of the early gastrula and neurula of *Triturus pyrrhogaster* was estimated by the technique of Schneider (1945) and expressed in microgram phosphorus per mg. nitrogen.

2. At both developmental stages PNA content was higher in the ectodermal regions than in the mesodermal regions. During gastrulation, an increase in PNA amount was noticed for most of the regions studied.

3. In the gastrula the highest amount of PNA was found in the ectoderm (prospective neural plate) but not in the organizer region. In the marginal zone the dorsal region showed an amount higher than that of the lateral and ventral marginal zones.

4. In the neurula the neural plate showed the highest value. The main part of the archenteric roof contained less PNA than the epidermis. No significant difference could be detected between the values of the archenteric roof and the rest of the mesoderm.

LITERATURE CITED

Brachet, J., 1941. La detection histochimique et le microdosage des pentose nucléiques. *Enzymol.*, **10**: 87–96.

Brachet, J., 1942. La localisation des acides pentose nucléiques dans les tissues animaux et les oeufs d'Amphibiens en voie de développement. *Arch. de Biol.*, **53**: 207–257.

Brachet, J., 1947. The metabolism of nucleic acids during embryonic development. *Cold Spring Harbor Symp.*, **12**: 18–27.

Levy, M., and A. H. Palmer, 1940. Iodometric estimation of small quantity of nitrogen without distillation. *J. Biol. Chem.*, **136**: 57–60.

Nakamura, O., 1942. Die Entwicklung der hinteren Körperhälfte bei Urodelen. *Annot. Zool. Japon.*, **21**: 170–235.

Okada, Y. K., and M. Ichikawa, 1947. Tables of developmental stages of *Triturus pyrrhogaster*. *Annual Rep. Exp. Morphol.* (*Tokyo*), 3: 1–6.

Schneider, W. C., 1945. Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. *J. Biol. Chem.*, **161**: 293–303.

Steinert, M., 1951. La synthèse de l'acide ribonucléique au cours du développement embryonnaire des batraciens. *Bull. Soc. Chem. Biol.*, **33**: 549–553.

Takata, K., 1950. On the distribution of RNA within the amphibian embryo. (In Japanese.) *Zool. Mag.* (*Tokyo*), **59**: 28.